

Article

MDPI

The delta subunit of rod-specific photoreceptor cGMP phosphodiesterase (PDE6D) contributes to hepatocellular carcinoma progression

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Supplementary Material









Figure 1. PDE6D-mediated ERK-activation in HCC. (A) Exemplary image (right side) and densitometric analysis (left side) of ERK-activation (pERK/ERK-levels) (Western blot analysis) in Hep3B cells after si-RNA-mediated PDE6D-suppression (*: p < 0.05 vs control). (**B**) Western blot analysis (exemplary image representing two independent experiments) revealing time-dependent recombinant Fibroblast-growth-factor (FGF)-induced ERK-activation (pERK/ERK-levels) in starved HCC cells (Hep3B) (cells were starved for 24 hours prior to stimulation with FGF) was prevented by co-treatment with the pharmacologic inhibitor of the PDE6D-KRAS-interaction deltarasin (5 μ M). (**C**) Phospho-ERK (pERK) and PDE6D levels after forced PDE6D overexpression in sorafenibresistant HCC cells (Hep3B).



Figure S2. Snail protein expression after TGF-β1-mediated stimulation of HCC cells. Densitometric analysis of Snail protein expression levels (Western blot analysis) in PLC cells that were stimulated with different doses (0, 1, 5 ng/ml) of recombinant human TGF-β1 for 72 hours, with or without co-treatment with 15 µM of the TGF-β-receptor-1 (TGFBR1) inhibitor LY2157299 ("galunisertib") (the densitometric values represent two independent Western blot analysis) (*: p < 0.05 vs control; ns: non-significant vs control).



Figure S3. KRAS expression and membrane localization in human HCC tissues as correlated with PDE6D expression and cytoplasmatic localization. (**A**) Tissue micro array analysis of PDE6D expression levels (high, medium, low) in human HCC tissues correlated with KRAS expression (high, low). (**B**) Tissue micro array analysis comparing KRAS membrane staining ("yes" vs "no") in human HCC tissues with ("yes") and without ("no") cytoplasmatic localization pattern of PDE6D.

					В		RABIS	
	loca	alization					T	
symbol	nuclear	non-nuclear	HCC-related (PMIDs)	Pathway/Function		RAP28	PICE	0
ARL15				Signaling, Ras-family		RHD		T
ARL2		3	27798868	Signaling, Ras-family			RADAS	
ARL3				Signaling, Ras-family		Levera I	RAPIA	RAB
CDC42			25978354	Cell cycle, mitosis			RHOA	3
CETN3				Cell cycle, mitosis			RAS GIAUT	
COPS5			27524414	Signaling	(1)	MAX UNC	HRIS	
CUL1			26097587	Protein degradation			e	
E2F1			28474358; 28134624	TF		URC		TGIR
FAM219A				nd		RADZ3A		
GNAI1			23691483	Signaling		Cur,	ARL3	
GRK1			20001100	Rhodonsin-signaling		CORE O		PDE6B
GRK7				Rhodonsin-signaling			Cemia 🔮	
HNF4A			29566023-28498607	TE		E2F1	ARL15 FALIZ194	PO
HRAS			25500025, 20450001	Cincoline				9
VDAC		_	29423069	Signaling				$\backslash $
KRAS			29275358	Signaling			and the second se	GRKT
MAX			29740493	1F				~
MYC			29893492	TF				- (
PDE6A				Rhodopsin-signaling				
PDE6B				Rhodopsin-signaling		KEGG Pathways		
PTGIR				nd	pathway ID	pathway description	count in gene set	false
RAB13				Signaling, Ras-family	04744	Phototransduction Chemoking ciacoling pathway	4	
RAB18			23471881	Signaling, Ras-family	04360	Axon guidance	5	
RABSA				Signaling, Ras-family	04010	MAPK signaling pathway	6	
				0		Federatesia	5	
RAD23A				Signaling, Ras-family	04144	Endocytosis		
RAD23A			27780720	Signaling, Ras-family	04144 05200	Pathways in cancer	6	
RAD23A RAP1A			27780730	Signaling, Ras-family Signaling, Ras-family	04144 05200 04014 05219	Pathways in cancer Ras signaling pathway Bladder cancer	6 5 3	
RAD23A RAP1A RAP2B			27780730 28081729	Signaling, Ras-family Signaling, Ras-family Signaling, Ras-family	04144 05200 04014 05219 04722	Pathways in cancer Ras signaling pathway Bladder cancer Neurotrophin signaling pathway	6 5 3 4	
RAD23A RAP1A RAP2B RASA1			27780730 28081729 26126858	Signaling, Ras-family Signaling, Ras-family Signaling, Ras-family Signaling, Ras-family	04144 05200 04014 05219 04722 05206	Protocyclosis Pathways in cancer Ras signaling pathway Bladder cancer Neurotrophin signaling pathway MicroRNAs in cancer	6 5 3 4 4	
RAD23A RAP1A RAP2B RASA1 RHEB			27780730 28081729 26126858 29467900	Signaling, Ras-family Signaling, Ras-family Signaling, Ras-family Signaling, Ras-family Signaling, Ras-family	04144 05200 04014 05219 04722 05206 05211	Endocytosis Pathways in cancer Ras signaling pathway Bladder cancer Neurotrophin signaling pathway MicroRNAs in cancer Renal cell carcinoma	6 5 3 4 4 3	
RAD23A RAP1A RAP2B RASA1 RHEB RHOA			27780730 28081729 26126858 29467900 29954442	Signaling, Ras-family Signaling, Ras-family Signaling, Ras-family Signaling, Ras-family Signaling, Ras-family Signaling, Ras-family	04144 05200 04014 05219 04722 05206 05211 05220 04750	Endocytistis Pathways in cancer Ras signaling pathway Biladder cancer Neurotrophin signaling pathway MicroRNAs in cancer Renal cell carcinoma Chronic myeloid leukemia Tölic hata signaling pathwar	6 5 3 4 4 3 3 3	
RAD23A RAP1A RAP2B RASA1 RHEB RHOA RHOB			27780730 28081729 26126858 29467900 29954442 28042950	Signaling, Ras-family Signaling, Ras-family Signaling, Ras-family Signaling, Ras-family Signaling, Ras-family Signaling, Ras-family	04144 05200 04014 05219 04722 05206 05211 05220 04350 04350	Endocytosis Pathways in cancer Pats signaling pathway Bladder cancer Neurotrophin signaling pathway MicroRNAs in cancer Penal cell cacicoma Chronic myebid leukemia TGF-beta signaling pathway Read signaling pathway	6 5 3 4 4 3 3 3 4	
RAD23A RAP1A RAP2B RASA1 RHEB RHOA RHOB RND1			27780730 28081729 26126658 29467900 29954442 28042950	Signaling, Ras-family Signaling, Ras-family Signaling, Ras-family Signaling, Ras-family Signaling, Ras-family Signaling, Ras-family Signaling, Ras-family	04144 05200 04014 05219 04722 05206 05211 05220 04350 04015 04510	Endocyclosis Pathways in cancer Ras signaling pathway Bladder cancer Neurotrophin signaling pathway Micro@NAs in cancer Renal cell carcinoma Chronic myeloid leukemia Chronic myeloid leukemia TGF-beta signaling pathway Rapt signaling pathway Rapt signaling pathway	6 5 3 4 4 3 3 3 4 4	
RAD23A RAP1A RAP2B RASA1 RHEB RHOA RHOB RND1 RPGR			27780730 28081729 26126858 29467900 29954442 28042950	Signaling, Ras-family Signaling, Ras-family Signaling, Ras-family Signaling, Ras-family Signaling, Ras-family Signaling, Ras-family Signaling, Ras-family Signaling, Ras-family	04144 05200 04014 05219 04722 05206 05211 05220 04350 04350 04310 05222	Endocyclosis Pathways in cancer Ras signaling pathway Bioder cancer Reard cell carcinomia Chronic myeloid leukemia Tóf-beta signaling pathway Rapt signaling pathway Focal adhesion Small cell lung cancer	6 5 3 4 3 3 3 3 4 4 4 3	
RAD23A RAP1A RAP2B RASA1 RHEB RHOA RHOB RHOB RND1 RPGR TAF1			27780730 28081729 26126858 29467900 29954442 28042950	Signaling, Ras-family Signaling, Ras-family Signaling, Ras-family Signaling, Ras-family Signaling, Ras-family Signaling, Ras-family Signaling, Ras-family Signaling, Ras-family Signaling, Ras-family Signaling, Ras-family	04144 0520 04014 05219 04722 05205 05211 05220 04350 04350 04350 04350 04351 05222 04350	Enlocyclosis Enlocyclosis Pathways in cancer Ras signaling pathway Bladder cancer Neurotrophin signaling pathway MicroRNAs in cancer Renal cell carcinoma Chonic myeloi leukemia TGF-beta signaling pathway Repat signaling pathway Repat signaling pathway Repat signaling pathway Resal adhesion Small cell lung cancer Pancreatic secretion	6 5 3 4 4 3 3 3 4 4 4 3 3 3 3 3 3 3 3 3 3	

Figure 4. Potential protein-interactions of PDE6D and PDE6D-interactome dependent pathways. (A) Analysis of protein-protein interaction datasets was performed using the "Harmonizome" database. The table depicts potential PDE6D-interacting proteins that were derived from low-throughput or high-throughput studies from the following databases: Reactome, NCI Pathways, PhosphoSite, HumanCyc, HPRD, PANTHER, DIP, BioGRID, IntAct, BIND, Transfac, MiRTarBase, Drugbank, Recon X, Comparative Toxicogenomics Database, and KEGG. The table also depicts information about cellular localization, HCC-relation according to literature (Pubmed-IDs) and related pathways/functions. The black arrows mark nuclear proteins. (B) String database and DAVID bioinformatics database-derived graphical illustration (top panel) as well as Gene enrichment based Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis (bottom panel) of the PDE6D-interactome.



Index	Name	P-value	Adjusted p- value	Z-score	Combined score
1	TP53	5.758e-8	0.00001226	-1.92	32.05
2	CCNE1	0.00001108	0.0007869	-1.49	16.99
3	CTNNB1	0.00005018	0.001165	-1.68	16.67
4	KAT2A	0.00003173	0.001116	-1.54	15.96
5	CCND1	0.00003291	0.001116	-1.53	15.79
6	CEBPA	0.00003931	0.001116	-1.53	15.55
7	SMAD4	0.00005512	0.001165	-1.58	15.53
8	HIF1A	0.00006016	0.001185	-1.45	14.07
9	MXI1	9.772e-7	0.0001041	-1.01	13.92
10	SMC3	0.00008331	0.001479	-1.35	12.64

P-value rank



MBD2

KAT5

CUL3 ATF2 NR3C E2F



Index	Name	P-value	Adjusted p- value	Z-score	Combined score
1	SMAD4	4.584e-11	6.152e-8	-1.80	42.94
2	TP53	2.944e-8	0.00001975	-1.70	29.50
3	SMAD2	5.922e-7	0.0001987	-1.78	25.52
4	SP1	5.922e-7	0.0001987	-1.76	25.28
5	MBD2	0.00001004	0.001684	-1.85	21.26
6	KAT5	0.00001004	0.001684	-1.72	19.81
7	CUL3	0.00001004	0.001684	-1.66	19.06
8	ATF2	0.00001004	0.001684	-1.64	18.91
9	NR3C1	0.0001408	0.007269	-1.85	16.40
10	E2F6	0.0001408	0.007269	-1.83	16.20
AD4					
P\$3					
MAD2					
1					

P-value rank

Figure S5. Potential transcription factor interactions of the PDE6D-interactome. Gene enrichment analysis using the "Enrichr" database depicting significantly enriched transcription factor terms for the 34 PDE6D-interacting protein list (Figure S1) in the "Enrichr Submissions TF-Gene Coocurrence" dataset (A) and the "Transcription Factor PPIs" dataset (B).